APPLIED MICROBIOLOGY, Oct. 1972, p. 567-570

Vol. 24, No. 4
Printed in U.S.A.

Introduction of *Pseudomonas aeruginosa* into a Hospital via Vegetables

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Received for publication 21 June 1972

Pseudomonas aeruginosa was isolated from tomatoes, radishes, celery, carrots, endive, cabbage, cucumbers, onions, and lettuce obtained from the kitchen of a general hospital, with tomatoes yielding both highest frequencies of isolation and highest counts. Presence of *P. aeruginosa* on the hands of kitchen personnel and cutting boards and knives which they used suggests acquisition of the organism through contact with these vegetables. It is estimated that a patient consuming an average portion of tomato salad might ingest as many as 5×10^3 colony-forming units of *P. aeruginosa*. Pyocine types of *P. aeruginosa* isolated from clinical specimens were frequently identical to those recovered from vegetables, thus implicating tomatoes and other vegetables as an important source and vehicle by which *P. aeruginosa* colonizes the intestinal tract of patients.

Pseudomonas aeruginosa is frequently involved in nosocomial disease infecting debilitated patients such as those with burns and those receiving antibiotics, cytotoxic or immunosuppressive therapy, as well as patients with indwelling catheters and those receiving respiratory assistance. Generally, the organism does not infect the healthy human. Although the organism colonizes the bowel of hospitalized patients at a high rate (11, 12), it is not commonly found in feces of normal individuals (6, 11, 12). In sporadic infections with a variety of types of *P. aeruginosa* occurring in patients on different wards (2, 8), the invading organism may have originated from the feces of patients acquiring *P. aeruginosa* in the intestinal tract after hospitalization.

Shooter et al. (10) examined cooked and uncooked foods in a hospital kitchen and found *Escherichia coli*, *P. aeruginosa*, and *Klebsiella* sp. and suggested that these organisms became established as intestinal flora of patients when ingested with such foods. Fresh vegetables, however, were not implicated as sources introducing *P. aeruginosa* into the hospital. We, therefore, proceeded to examine uncooked vegetables in the kitchen of our hospital to determine whether these represented sources of *P. aeruginosa*.

MATERIALS AND METHODS

Collection of vegetable samples. From January 1972 through May 1972, 188 samples of vegetables and vegetable salads were examined for recovery of *P. aeruginosa*. Samples weighing from 50 to 300 g were obtained from the hospital kitchen during the morning hours of 9 to 11 AM while vegetables and salads were under preparation. Tomato samples consisted of 2 to 3 tomatoes except when in the form of salads. All vegetables had been rinsed with tap water by kitchen personnel and set in large colanders until cut for distribution. Vegetables were obtained after cutting but prior to preparation for distribution, whereas individual salad portions were obtained after preparation and prior to distribution to wards.

Vegetables tested in this study were shipped from Mexico, Southern California, and Louisiana. No determination was made, however, as to the relationship, if any, between geographical origin of the vegetables and frequency of recovery of *P. aeruginosa*.

Isolation of P. aeruginosa from vegetables. All samples were cultured within 3 hr after collection. They were weighed and then homogenized with a Waring blendor for 30 to 60 sec. In order to facilitate homogenization, sterile distilled water was added in equal volumes to the weight of each sample of all vegetables except tomatoes. The final colony count per milliliter of homogenate was doubled to account for this dilution factor.

Surface streak cultures of the homogenate were prepared by plating 0.1 ml on cetrimide-agar (0.3% cetrimide in Mueller-Hinton agar, Difco). In all, eight plates were cultured from each sample. At the same time, two tubes of 5 ml of Trypticase soy broth (BBL) containing nitrofurantoin at 0.2% were inoculated with 0.5 ml of homogenate.

All cultures were incubated at 42 C, and the plates were examined with a Wood's lamp for fluorescence after 24 and 48 hr. Final counts were taken

when fluorescence was easily observed. Broth cultures were incubated for 48 hr at 42 C, subcultured onto cetrimide-agar, and incubated at 42 C for 18 hr before examination under ultraviolet light.

Colonies on cetrimide-agar with yellow-green or blue-green fluorescence under ultraviolet light were suspected to be *P. aeruginosa*. For identification, in addition to growth on cetrimide-agar at 42 C, fluorescence, and pigment production, three additional tests were performed on one to five colonies from each sample: oxidase, motility, and oxidation of glucose (3). Positive reactions in each of these confirmed the colonies as *P. aeruginosa*.

No efforts were made to count or identify nonfluorescent colonies from the vegetable samples growing on cetrimide-agar.

Cultures of personnel and environment. Hands of employees preparing and portioning vegetables and salads were cultured by impression of palms and fingers of each hand on a rodac plate with cetrimide-agar. Cutting boards on which vegetables were sliced and knives used for this purpose were also examined by impression on rodac plates.

The plates were incubated for 48 hr at 42 C and fluorescent colonies were identified as *P. aeruginosa* according to the stated criteria.

Pyocine typing. From each sample of vegetables, a maximum of 20 colonies of *P. aeruginosa* were pyocine typed. All isolates from environmental and personnel sampling in the kitchen and all cultures of *P. aeruginosa* obtained in the clinical bacteriology laboratory during the period of this study (January 1972 through May 1972) were pyocine typed. A total of 713 isolates were typed by the pyocine production method.

In principle, a pyocine-producing isolate of *P. aeruginosa* is recognized as a certain type by the pattern of the inhibition produced using 11 indicator strains. The procedure used is presented in detail by Zabransky and Day (13).

RESULTS

P. aeruginosa was recovered in 82% of whole tomato samples and in 27% of tomato salads as shown in Table 1. *P. aeruginosa* was present in all vegetables, but the tomatoes yielded both higher frequencies of isolation and higher counts than other vegetables. Thus, 64% of the whole tomatoes which were positive yielded more than 100 colony-forming units of *P. aeruginosa*/ml of homogenate (with one sample having as many as 2.2×10^3/ml); 33% of the tomato salads which were positive carried more than 100 colony-forming units/ml. Also, more than 100 colony-forming units of *P. aeruginosa*/ml were present in at least one sample each of radishes, celery, and carrots, whereas less than 100 colonies/ml were recovered from samples of endive, cabbage, cucumbers, onions, and lettuce.

Table 2 shows the pyocine types of *P. aeruginosa* isolated from vegetables. The most common types were F-6, D-2, B-7, and F-2. Pyocine typing of the clinical isolates (Table 3) revealed many of the types to be the same as those recovered from vegetables. In fact, four of the six most frequent types from clinical specimens were the same as those from vegetables: B-7, F-6, D-2, and F-2. Two additional types, B-3 and E-6, although very common among clinical isolates, were less frequently recovered from vegetable samples. Whereas 93% of the strains from clinical specimens were typable by the pyocine method, only 84% of the vegetable isolates were identifiable as types.

P. aeruginosa was found on the hands of 5 of 52 of the kitchen personnel who were cultured while cutting and portioning vegetables as well as on some of the boards and knives which they used for salad preparation (Table 4). The pyocine types encountered were the same as those recovered from vegetables and from some clinical cases (Tables 2 and 3). *P. aeruginosa* was also recovered from unwashed samples of vegetables obtained directly from the delivery truck prior to contact by the kitchen personnel.

DISCUSSION

P. aeruginosa was found in various vegetables in the hospital kitchen, with highest counts recovered from tomatoes and tomato-containing salads. Its presence on the hands of personnel preparing and portioning vegetables and salads and on the knives and cutting boards which they used, in addition to its recovery from vegetables obtained upon delivery prior to personnel handling, suggests that the

TABLE 1. *Isolation of Pseudomonas aeruginosa from vegetables*

Vegetable	Samples		No. of samples with indicated colony counts[a]			
	No. cultured	No. positive	10^0 to 10^1	10^1 to 10^2	10^2 to 10^3	10^3 to 10^4
Tomatoes	17	14	3	2	7	2
Radishes	9	7	3	2	2	
Celery	6	4	2	1	1	
Carrots	10	3	2		1	
Endive	8	2	1	1		
Cabbage	7	2	1	1		
Cucumbers	6	2	2			
Onions	5	1	1			
Lettuce	9	1	1			
Tomato (salad)	111	30	16	4	10	

[a] Per milliliter of homogenate.

TABLE 2. *Pyocine types of Pseudomonas aeruginosa isolated from vegetables*

Vegetable	Pyocine types											
	B-3	B-4	B-6	B-7	D-2	E-6	E-7	F-2	F-6	J-6	VT[a]	NT[b]
Tomatoes		2	7	21	39			10	33		14	21
Radishes				3	12						37	5
Celery	6		2		12				10		3	4
Carrots			2	2	1	2	5	3	9		2	3
Endive					3						1	1
Cabbage											1	4
Cucumbers				9							1	2
Onions					1							1
Lettuce									1			
Tomato (salad)	3			1	19	1	4	7	40	2	13	25

[a] VT, Variable type: unstable typing pattern.
[b] NT, Nontypable: no inhibition of indicator strains.

TABLE 3. *Pyocine types of Pseudomonas aeruginosa isolated from clinical specimens*

Specimen	Pyocine types															
	B-3	B-4	B-5	B-6	B-7	D-2	E-6	E-7	F-2	F-4	F-5	F-6	J-2	J-6	VT[a]	NT[b]
Urine	10	3	4	4	16	10	4	2	7	3		17	4	1	14	9
Sputum	2	1		3	9	14	7	1	8	4	4	6		1	16	6
Wound[c]	6			1	16	10	7	1	3	3	2	11		2	11	3
Feces	1				2	1				1					3	
Blood	2				1		1							1		2

[a] VT, Variable type: unstable typing pattern.
[b] NT, Nontypable: no inhibition of indicator strains.
[c] Wounds are either postoperative or burns.

organism may have been acquired from the vegetables.

The common pyocine types of our clinical specimens (B-7, D-2, F-2, and F-6) were generally identical to those recovered from vegetables. The sharing of pyocine types between vegetable isolates and those from clinical specimens supports the hypothesis that vegetables, and especially tomatoes, constitute an important primary source of *P. aeruginosa*. This organism, ingested with vegetables, may colonize the intestinal tract of hospitalized patients. In addition, in susceptible individuals, intestinal colonization may in turn lead to infection, complicating their underlying disease with subsequent transmission of the organism to other patients via the hands of nursing personnel (5, 7).

Tomato salads carry an average of 70 colony-forming units of *P. aeruginosa*/ml of homogenate. Therefore, a patient who consumed 80 g of salad (average portion) ingested as many as 5×10^3 colony-forming units of *P. aeruginosa*. However, it is conceivable that not all patients eating vegetable salads ingested the organism in such large numbers. A lower dose may also lead to intestinal colonization due to the

TABLE 4. *Isolation of Pseudomonas aeruginosa from personnel and environment in kitchen*

Specimen	No. cultured	No. positive	Pyocine types
Personnel hands	52[a]	5	B-7[b], F-6, NT[c]
Tomato cutting board	27	13	B-7[b], D-2, D-5, F-2, F-4, F-6, R[b], NT[b]
Tomato knife	3	0	
Radish knife	1	1	F-6
Carrot cutting board	3	0	
Carrot knife	2	1	E-7
Cabbage knife	5	1	D-2
Cucumber cutting board	1	0	
Cucumber knife	1	0	
Onion cutting board	1	0	
Onion knife	2	1	D-2
Lettuce cutting board	3	0	
Lettuce knife	1	0	

[a] Number represents individuals; both hands examined.
[b] Multiple recovery.
[c] NT, Nontypable: no inhibition of indicator strains.

greater resistance of *P. aeruginosa* (compared to enteric bacteria) to antibiotics and chemotherapeutics (4). The minimum number of *P. aeruginosa* required for colonization in the bowel of patients receiving antibiotics was not reported.

Buck and Cooke (1) showed experimentally that ampicillin treatment prolonged fecal carriage of ingested *P. aeruginosa* beyond the average 48- to 72-hr period seen in normal individuals. Antibiotic administration with concurrent ingestion of *P. aeruginosa* in vegetables may facilitate the intestinal colonization of hospitalized patients with this organism. Namely, the vehicle of food very likely contributes to the higher rate of fecal carriage of 18 to 20% in hospitalized patients (11, 12) as compared to that of 3 to 6% observed in normal individuals (6, 11, 12). Attempting to explain this high fecal carriage in patients, Shooter et al. (10) recovered *E. coli*, *P. aeruginosa*, and *Klebsiella* sp. from various foods in the hospital kitchen and hypothesized that ingestion may lead to colonization.

Cross-contamination of other foods in the kitchen could possibly take place since cutting boards and knives used for slicing vegetables were also contaminated. Other foods prepared on these boards or with the same knives could easily acquire the organism, but this vehicle remains to be investigated.

The work of Samish and Etinger-Tulczynska (9) suggests that *P. aeruginosa* is an epiphyte which colonizes tomatoes. *Pseudomonas* was found mostly in the stem scar and the underlying core of healthy tomatoes along with *Xanthomonas*, *Enterobacteriaceae*, and *Corynebacteriaceae*. By using pigmented *Serratia marcescens* as an indicator, the stem depression was shown by these authors to be the point of entry into the tomato (9). It is not documented, however, whether *P. aeruginosa* is acquired from soil, fertilizers, or water used for irrigation.

ACKNOWLEDGMENTS

We thank Ann Hunt and dietary personnel of Mercy Hospital for their invaluable assistance in obtaining vegetable samples. We also thank the medical technologists in the hospital bacteriology laboratory for providing cultures of *P. aeruginosa* from clinical specimens.

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